

CHLORIDE IONS AND THE MEMBRANE POTENTIAL OF PURKINJE FIBRES

By E. E. CARMELIET*

From the Department of Physiology, University of Berne, Switzerland

(Received 12 December 1960)

According to the ionic theory of electrical activity the membrane potential depends on the unequal distribution of ions between the intra- and extracellular phases, and on the relative permeability of the membrane towards them. For cardiac muscle the role played by sodium and potassium has been studied to some extent (see Hoffman & Cranefield, 1960) but that of chloride has received little attention. The present paper deals with the effects produced by the substitution for chloride ions of nitrate ions or large anions such as acetylglycinate and pyroglutamate. The results suggest that the contribution of chloride ions to the total membrane conductance is low in a resting fibre but increases when a fibre is depolarized. The findings and interpretations are in good agreement with those recently published by Hutter & Noble (1959). The main results of the present investigation have been reported in a preliminary communication (Carmeliet, 1959).

METHODS

Sheep hearts were obtained at the slaughterhouse and carried to the laboratory in cool Tyrode solution. 'False tendons' were removed from the left ventricle and kept in Tyrode solution at 37° C.

Membrane potentials were measured by two Ling-Gerard micro-electrodes (1949), one inside, the other outside the fibre. The electrodes were filled with 3M-KCl and had a resistance between 10 and 20 MΩ. They were connected to the cathode-follower input of a differential amplifier by chlorided silver wires.

In a series of experiments the relative membrane resistance was measured by passing current through a second micro-electrode within the same fibre. Square current pulses of about 100 msec duration were used which hyperpolarized the membrane, at a frequency of 4/sec. The distance separating the intracellular electrodes was small (50–100 μ) compared to the space constant of a Purkinje fibre (2 mm; Weidmann, 1952). The amplitude of the recorded potential changes could thus be squared to obtain relative values for the membrane resistance (Hodgkin & Huxley, 1947).

In order to make measurements of membrane resistance at different membrane potentials the fibre was depolarized or hyperpolarized by means of a pair of external electrodes, as shown in Fig. 1. The bundle was pushed into a close-fitting hole in a Perspex block and solution was allowed to siphon along this part of the preparation. The two micro-electrodes were inserted at a distance of 1 mm from the hole. To insure that the recording micro-

* Present address: Department of Physiology, University of Louvain, Belgium.

electrode was measuring the true membrane potential change, the reference electrode was so placed that with the recording micro-electrode just outside the fibre there was no potential change when current was switched on.

The composition of the normal Tyrode was as follows (mm): Na 149.9; K 2.7; Ca 1.8; Mg 0.5; Cl 145; HCO_3 11.9; H_2PO_4 0.32; glucose 1 g/l. The solution, when aerated by 95% O_2 and 5% CO_2 , had a pH of 7.4. To study the influence of anions, NaCl was replaced by Na-acetylglycinate (Light) or Na-pyroglutamate (Light), or NaNO_3 . The K^+ concentration was reduced by omission of KCl, and increased up to 50 mM by replacement of an equivalent amount of the NaCl. In sodium-free solutions, NaCl was replaced by choline chloride (Hoffmann-La Roche) on a mole-for-mole basis, and atropine sulphate was added (10 mg/l.). Choline acetylglycinate was used as a substitute for NaCl, in an attempt to combine a slowly penetrating cation with a slowly penetrating anion. This was prepared by neutralizing acetylglycine with choline hydroxide (Schuchardt). Choline nitrate was prepared from choline chloride, by adding AgNO_3 in an amount slightly less than equivalent, and filtering.

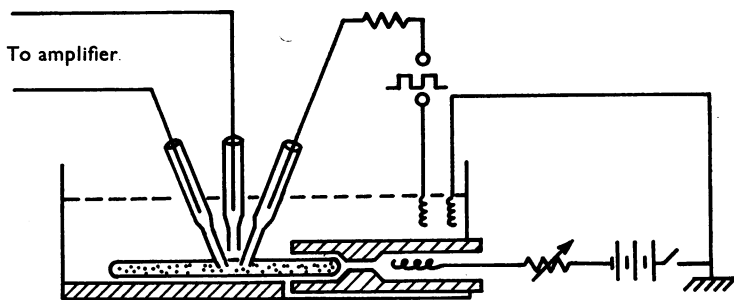


Fig. 1. Diagram of arrangement to measure the relative membrane resistance at different membrane potentials. Two intracellular micro-electrodes, one to record the membrane potential, the other to pass square wave current pulses of about 100 msec duration. The bundle was pushed into a close-fitting hole in a Perspex block, which served as an external polarizing electrode.

RESULTS

Effects of replacing chloride ions by other anions

The substitution of NaCl by Na-pyroglutamate or Na-acetylglycinate had the following effects (Figs. 2 and 3): (a) the frequency of spontaneously beating preparations went up temporarily, owing to a higher rate of the diastolic depolarization; (b) spontaneous activity often started with preparations that had previously been quiescent; (c) the plateau of the action potential was markedly prolonged; its slope was decreased, sometimes resulting in a standstill on the plateau level (-50 mV); (d) the plateau was also elevated, and this was most pronounced if the preparation was artificially stimulated at a high rate.

The substitution of chloride ions by nitrate ions produced the opposite effects: (a) the frequency of spontaneous activity was reduced, owing to a lower rate of the diastolic depolarization; (b) at the end of 5–10 min spontaneous activity ceased, and the fibre depolarized by about 10 mV,

to reach a stable membrane potential near -80 mV; (c) the plateau of the action potential was shortened, and (d) was brought closer to the resting potential (see Fig. 3). On returning from nitrate to chloride solution the action potentials initially lengthened beyond their previous duration to return to 'normal' with a half time of about 5 min.

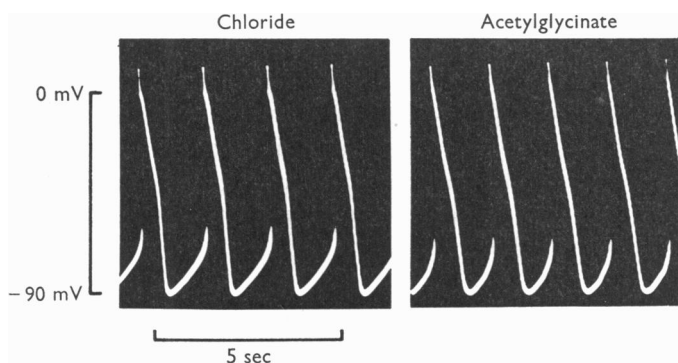


Fig. 2. Spontaneous action potentials in chloride-Tyrode (left) and in acetyl-glycinate-Tyrode (right). The rate of diastolic depolarization increased in the acetyl-glycinate solution.

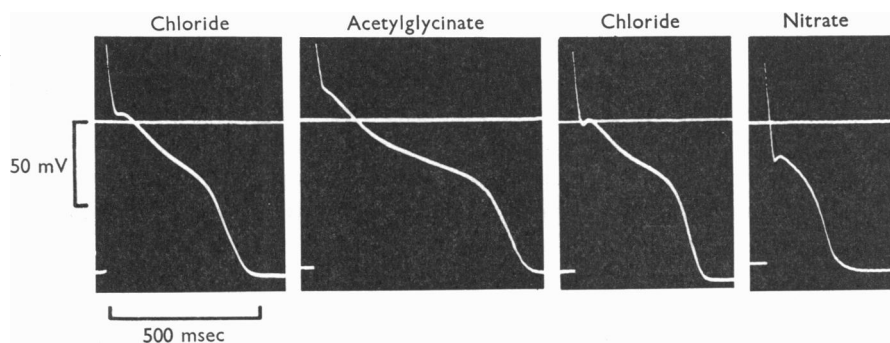


Fig. 3. Action potentials in chloride-Tyrode are compared with action potentials in acetyl-glycinate and nitrate solutions. The spike has been retouched.

In contrast to the considerable influence of anions on the shape of the action potential, substitution of chloride by large anions had no effect on the value of the maximum diastolic potential. To eliminate complications due to spontaneous activity chloride substitution was also studied in the absence of Na ions. Replacement of choline chloride by choline acetyl-glycinate did not change the value of the resting potential. This remained true also if the potassium concentration was increased from normal (2.7 mM) to 5.4, 13.5 or 50 mM.

For the sake of simplicity in the presentation of further results it may now be helpful to put forward a possible explanation. It is assumed (i) that

the membrane is permeable to anions, the permeability decreasing according to the series $\text{NO}_3 > \text{Cl} > \text{acetylglycinate}$; (ii) that the permeability to other ions is relatively large at the resting potential, but becomes comparable to that of chloride ions when the membrane is depolarized; and (iii) that chloride ions are passively distributed between the intra- and extracellular phases.

With this hypothesis in mind the observed effects may be explained in the following way. When the membrane is depolarized there is an inward current of chloride ions. This will tend to make the outside of the membrane more positive, and thus assists repolarization. With nitrate replacing chloride, this current will be increased, resulting in a shorter action potential and in a lower rate of diastolic depolarization. In the acetylglycinate solution the opposite effect will occur.

Chloride substitution in a potassium-free medium

In order to test the assumption that chloride ions play an important part in carrying electric charge when the membrane becomes depolarized, the effect of chloride substitution was studied in a potassium-free medium. While myocardial fibres hyperpolarize or do not change their membrane potential if potassium ions are omitted from the bathing solution, Purkinje fibres show a strong depolarization to a level near -50 mV (Weidmann, 1956). Flux measurements with ^{42}K indicate a decrease of potassium permeability (Carmeliet, 1960); Na ions are necessary to obtain the depolarization. So we have to deal with a situation in which potassium permeability is decreased, whilst the sodium permeability is appreciable. The potential in a K-free solution may be stable at -40 mV or may oscillate between -50 and 0 mV.

When chloride ions were replaced by acetylglycinate there was a further tendency for the membrane to depolarize: the oscillations became larger and more frequent; they would continue or disappear after some time. The resemblance to the influence of chloride substitution on the diastolic depolarization was obvious. On returning to the chloride-Tyrode solution without potassium, the oscillations disappeared and the membrane potential returned slowly towards -50 mV. Then suddenly, within a fraction of a second, the potential returned to -90 mV (Fig. 4). This full repolarization was followed by a train of spontaneous action potentials of normal configuration, but with increasing duration, until the membrane failed to repolarize and the potential became stable again at the -50 mV plateau level.

These results may be explained on the three assumptions stated above. Thus, when the fibre is depolarized in a K-free solution, the chloride ions redistribute according to the new potential. If chloride ions are now

replaced by acetylglycinate ions the outflow of chloride ions outweighs the inflow of the larger anions. The net outflow of negative charge explains the larger and more frequent oscillations. Readmitting the chloride ions results in an inward flow of negative charge, thus the tendency to repolarize. However, the potential returns to the plateau level (-50 mV) as soon as the internal chloride concentration has risen sufficiently to slow down the net entry of chloride ions. It is noteworthy that readmission of chloride

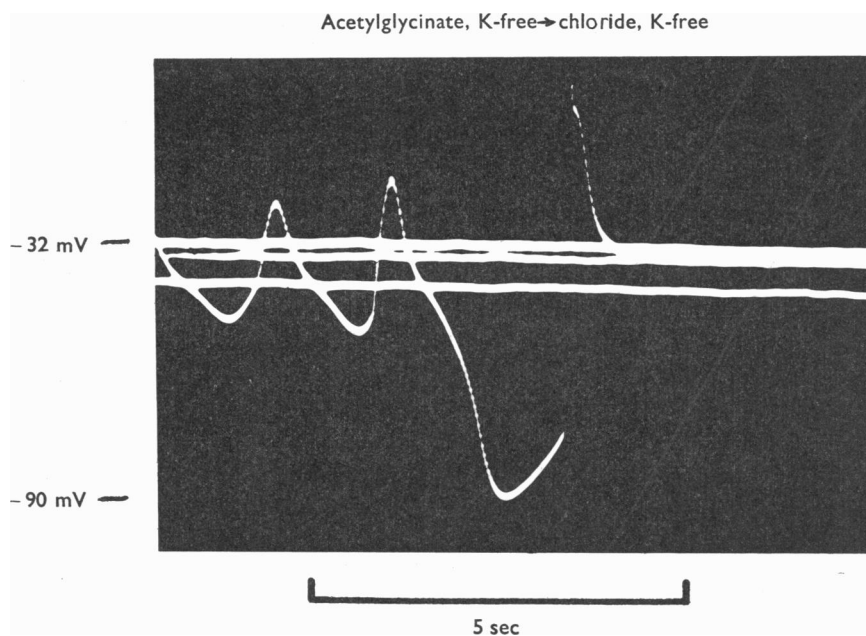


Fig. 4. Effect of replacing acetylglycinate by chloride ions in a potassium-free medium. The membrane was first depolarized in a potassium-free acetylglycinate medium to -32 mV. Readmitting chloride ions brought about a transitory repolarization to -90 mV.

ions has a larger effect than chloride deprivation. This may be explained by the difference in the relative change of the chloride concentration gradient and by regenerative phenomena: during the flow of hyperpolarizing current a decrease in sodium permeability could be coupled with an increase in potassium permeability, both favouring repolarization.

While nitrate ions hardly increased the resting potential when the normal potassium concentration was used, they caused a hyperpolarization of more than 20 mV if the membrane was first depolarized by potassium lack. Reverting to the normal chloride concentration resulted in a marked depolarization. This again can be explained by a larger permeability to nitrate than to chloride ions.

Chloride substitution in a potassium- and sodium-free solution

H. H. Hecht & S. Weidmann had found (unpublished observations) that Purkinje fibres in a sodium- and potassium-free solution could show two different stable potentials, -90 and -50 mV. When potassium was omitted first there was a depolarization to -50 mV, and the reduction of sodium ions did not change the potential further. When sodium was omitted first, the potential stayed near -90 mV, and a decrease in the potassium concentration had no effect (Fig. 5).

The following experiments were undertaken to find out whether the influence of the sequence of potassium and sodium deprivation on the final result depended on the stabilizing effect of the chloride ions. In a first series of experiments the 'chloride battery' was rendered less important by working in a Cl-free (acetylglycinate) medium. Removal of the potassium ions led to depolarization, as usual. When sodium ions were now omitted and, simultaneously, the chloride concentration was restored to normal, the potential returned to -90 mV (Fig. 6). If the chloride ions had been readmitted in the presence of sodium ions the repolarization would have been transitory (see preceding section). In the absence of inward current due to the sodium ions, however, the potential became stable at the high value.

In a second series of experiments chloride ions were replaced by nitrate ions. The sequence is illustrated in Fig. 6. A Purkinje fibre was first depolarized in a potassium-free solution. Replacing the NaCl by choline nitrate caused a marked and stable repolarization.

The main result of these experiments is: reduction of sodium ions alone does not change the potential of the fibre depolarized by potassium lack to any large extent. If, however, at the same time there exists a disequilibrium for the anion distribution, the membrane can be fully repolarized. The permeability to anions in the depolarized state seems to be relatively important.

Polarizing current in a potassium- and sodium-free solution

If the chloride ions really distribute themselves passively it ought to be possible to change the intracellular concentration by altering the membrane potential for some time. After the break of current applied from an external source, the membrane potential should have a tendency, thanks to the redistribution of the chloride ions, to stay near the value that has been imposed.

The membrane potential was changed by means of current flowing through an intracellular electrode; the potential change was recorded by a second intracellular electrode. In order to obtain a fairly uniform

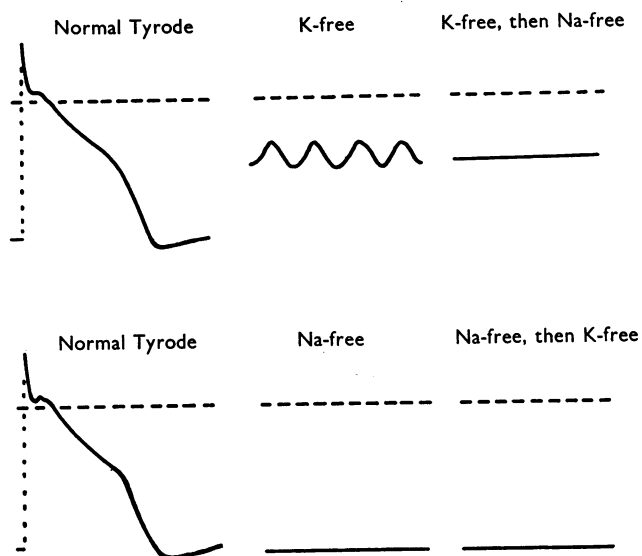


Fig. 5. Tracings of the transmembrane potential of a sheep Purkinje fibre: the sequence of potassium and sodium depletion determined the potential finally obtained.

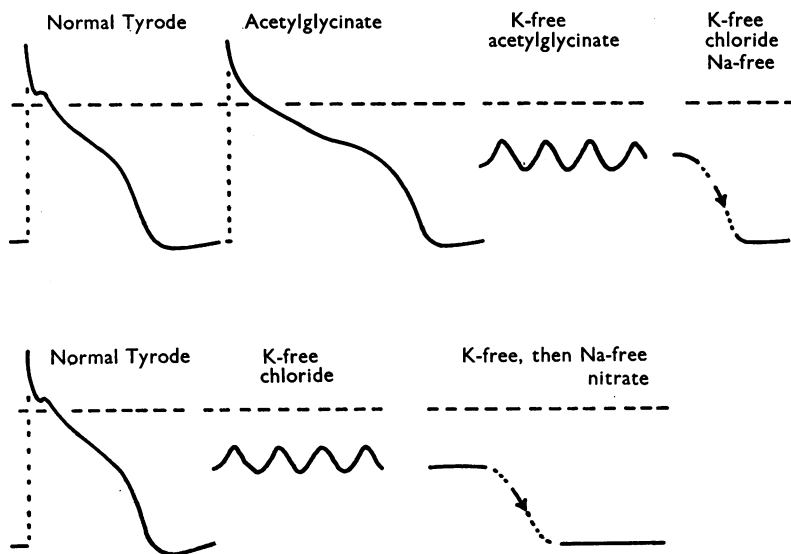


Fig. 6. Tracings of the membrane potentials of a sheep Purkinje fibre: influence of the disequilibrium for anions in a potassium- and sodium-free medium. For explanation see text.

change over the whole surface of the membrane it was necessary to use short pieces (order of one space constant, $= 2$ mm). If any biological cable is cut transversely, there is a depolarization, which is most pronounced at the cut surface but spreads along the membrane for some distance due to the fact that the extracellular fluid and the 'core' represent a low resistance to the flow of short-circuit currents. In an empirical way it was found that short pieces with 'seals' at both ends could best be obtained if the second cut was made in a sodium-free and potassium-rich (13.5 mM) medium. In practice it was possible to obtain pieces of Purkinje fibres 3 mm in length which, when transferred to Tyrode solution and stimulated, showed normal action potentials.

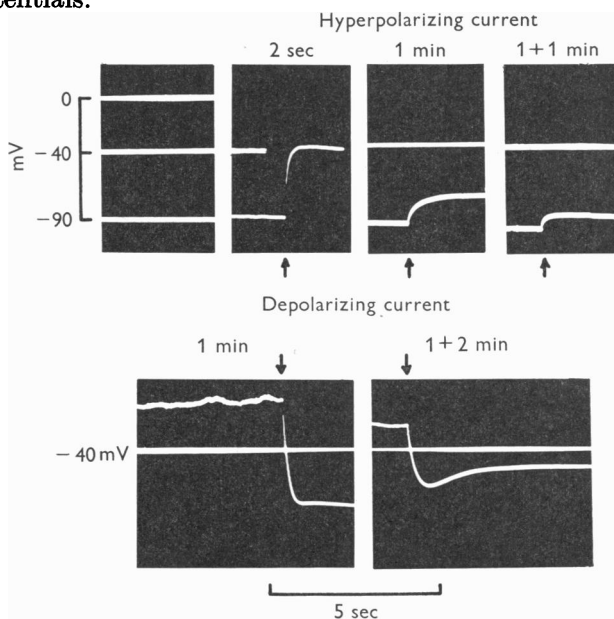


Fig. 7. Effect of polarizing current in a potassium- and sodium-free solution. Above (from left to right): the two possible membrane potentials in a potassium- and sodium-free medium (-40 and -90 mV); effect on the membrane potential of a repolarizing current of 2 sec, 1 min and 1+1 min duration. Below: effect of a depolarizing current of 1 min, and 1+2 min duration. Arrows indicate break of current.

A typical experiment will be described. Two micro-electrodes were inserted, one to record, the other to polarize. First the potassium was reduced, afterwards the sodium. The result was a depolarization to -40 mV. Figure 7 illustrates the effects of then applying hyperpolarizing currents for different periods. At the break of a hyperpolarizing current lasting for 2 sec the potential returned quickly to the -40 mV level. After passing a hyperpolarizing current for 1 min, however, the potential

did not return to the initial level but stayed at an intermediate value. A hyperpolarizing current reapplied for a further minute resulted in a full and stable repolarization to -90 mV. Soon afterwards current was passed in the opposite direction. A depolarizing current lasting for 1 min caused only a slight reduction in potential below -90 mV. Having passed current for another 2 min the potential stayed at the -50 mV level.

It is thus possible to move the potential from one stable level to the other, by imposing on the membrane that new potential value for a few minutes. The current must be carried by ions that are present within the intra- and extracellular phases. An inward current must be carried by positive ions moving inwards or by negative ions moving outwards. There was no sodium or potassium in the extracellular solution. The only ions moving inwards could be choline, magnesium or calcium, but it is hard to see how an inward flux of these ions could stabilize the potential at the -90 mV level. However, it seems reasonable to suggest that the current is carried by Cl ions moving outwards. With a decrease of the internal chloride concentration the chloride equilibrium potential would tend to approach the -90 mV level.

The effect of anions on the membrane resistance

A change in the electrical membrane resistance when an ion concentration is altered may indicate the importance of that particular ion as a carrier of membrane current. It had been reported in an abstract by Weidmann (1955) that the electrical membrane resistance of Purkinje fibres, measured 2 sec after the make of a polarizing current, was a function of the membrane potential. To simplify the system, sodium-free solutions were used in the present study. As can be seen in Figs. 8 and 9 the resistance was maximal between -20 and -40 mV. For reversed membrane potentials the resistance decreased to about quarter of its maximum value near $+40$ mV. The resistance at -90 mV depended strongly on the external potassium concentration and could be smaller (high potassium concentration) or larger (low potassium concentration) than the value at $+40$ mV.

The effect of the chloride concentration was studied at different potassium concentrations (0, 2.7, 5.4 and 50 mM). Chloride was replaced by acetylglycinate or nitrate. The results were dependent on the potassium concentration. At potassium concentrations of 0, 2.7 and 5.4 mM, replacement of chloride by acetylglycinate resulted in an increase of the membrane resistance if the membrane was depolarized beyond -50 mV. Figure 8 shows an example at 0 mM potassium. The increase was to about twice for potentials between 0 and $+50$ mV. At potentials between -50 and -100 mV it was hard to see any difference. In some experiments there was a slight increase of resistance in the acetylglycinate solution, but it

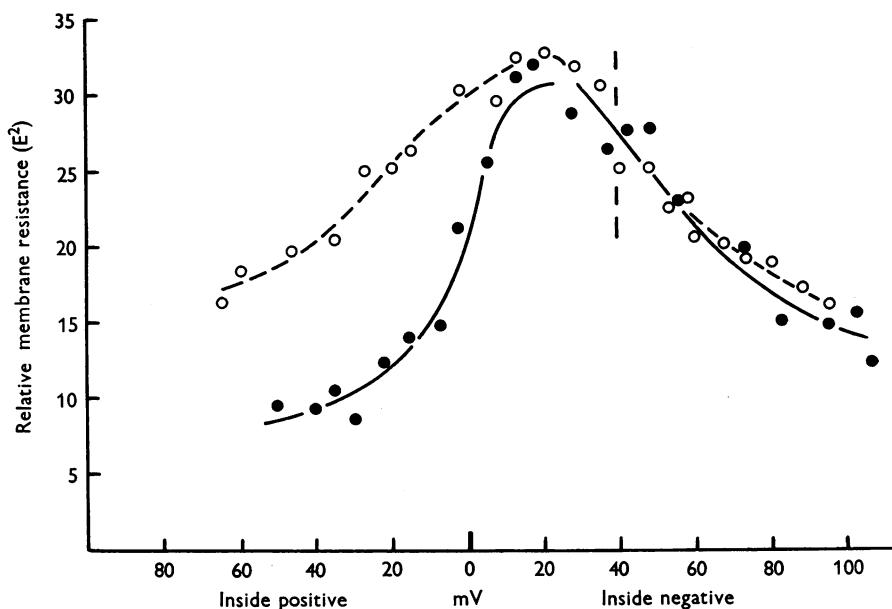


Fig. 8. Relative membrane resistance as a function of the membrane potential. ●, chloride medium; ○, acetylglycinate medium. The vertical interrupted line indicates the membrane potential without polarizing current; the extracellular potassium concentration was 0 mM. For large depolarizations (–20 to +60 mV inside potential), the membrane resistance in the acetylglycinate medium was higher than in the chloride medium.

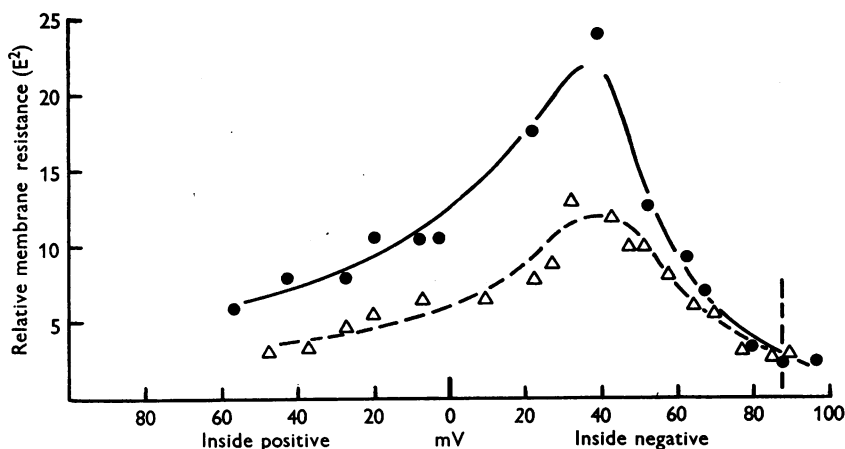


Fig. 9. Relative membrane resistance as a function of the membrane potential. ●, chloride medium; △, nitrate medium. The vertical interrupted line indicates the membrane potential without polarizing current; the extracellular potassium concentration was 5.4 mM. Between –40 and +60 mV the resistance was decreased in the nitrate-Tyrode. Note that the resistance at –90 mV was lower than at +40 mV, while the reverse was true in Fig. 8. This is due to the difference in potassium concentration.

was impossible to be sure that these differences were not due to regenerative phenomena or to small differences in the estimated membrane potential.

The reverse result was observed in the nitrate solution (Fig. 9, potassium concentration 5.4 mM). The membrane resistance was roughly halved between -20 and $+50$ mV, but was unchanged between -50 and -100 mV.

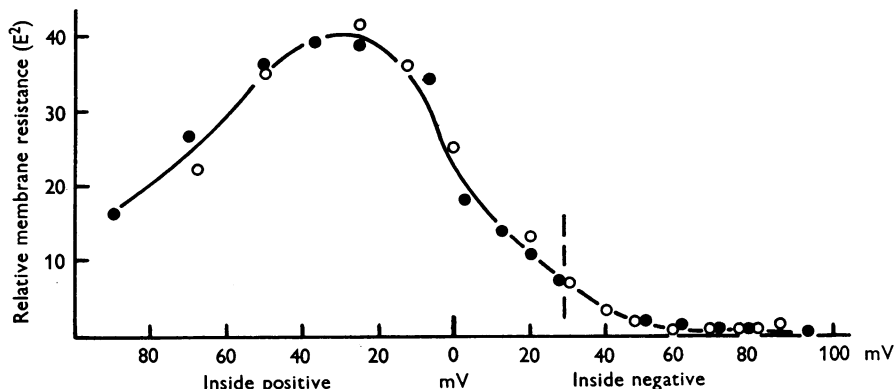


Fig. 10. Relative membrane resistance as a function of the membrane potential. ●, chloride medium; ○, acetylglycinate medium. Vertical interrupted line, membrane potential without polarizing current. Potassium concentration was 50 mM. There was no difference between the two curves.

At a potassium concentration of 50 mM there was no difference at any membrane potential between the chloride and the acetylglycinate solution (Fig. 10). It might be stressed that the anomalous rectification found in the normal Tyrode solution was also present in high potassium concentrations, and was even more pronounced.

The conditions under which chloride ions become important as carriers for current are thus: (a) depolarization, (b) a low external potassium concentration.

DISCUSSION

From the point of view of describing electrical events during the cardiac cycle, the conclusion of the present experiments is thus: in resting fibres, chloride ions play but a minor role; however, when the fibres are depolarized, as during activity, chloride ions become important as carriers of electrical charge. This seems to be due to a decrease in K conductance of the surface membrane upon depolarization rather than to an increase in Cl conductance.

The question as to whether or not chloride ions are distributed as 'passive' ions between the intra- and the extracellular phases cannot be

answered in a final way. The intracellular Cl concentration corresponding to a resting potential of -90 mV and an extracellular Cl concentration of 145 mM would be 5 mM. Analytical data for intracellular chloride are difficult to obtain since the methods depend on a correct estimation of the extracellular space. It is felt that the analytical figures so far published cannot be used as evidence either for or against the idea of a passive distribution (Robertson & Peyser, 1956; Lamb, 1960). It was reported in the present paper that the membrane potential can be permanently displaced (p. 380) by imposing a new potential on the membrane for a few minutes. This result can easily be explained by assuming a passive redistribution of chloride, but does not provide final evidence.

Tracer data indicate that chloride exchange in rabbit heart is relatively quick (order of a few minutes, Manery & Haege, 1941; Sekul & Holland, 1959). The question thus arises why in a resting fibre chloride was found to contribute so little to the membrane conductance. The apparent difficulty finds an explanation if the assumption may be made that the intracellular chloride concentration is low (near 5 mM). Thus a relatively high rate constant would be compatible with a small contribution of chloride ions as carriers of electrical charge.

Finally, it seems worth while to compare some membrane properties of skeletal and cardiac muscle. In both tissues inward movement of chloride ions during the action potential has been shown to contribute to repolarization (Etzensperger & Bretonneau, 1956; Falk & Landa, 1960; Hutter & Noble, 1960). On the other hand a number of differences exist: (a) for a resting membrane the chloride conductance is relatively small in cardiac tissue and large in skeletal muscle (Giebisch, Kraupp, Pillat & Stormann, 1957; Hodgkin & Horowicz, 1959; Hutter & Padsha, 1959); (b) this difference is still present when the extracellular potassium concentration is increased (Hodgkin & Horowicz, 1959; Adrian, 1960); (c) nitrate ions penetrate more slowly than chloride ions in skeletal muscle (Hutter & Padsha, 1959), while this order is reversed in cardiac muscle.

SUMMARY

1. Na acetylglycinate, Na pyroglutamate and NaNO_3 were used as substitutes for NaCl in order to study the influence of anions on the membrane potential and membrane resistance of Purkinje fibres of sheep hearts.

2. The anions had no influence on the maximum diastolic potential, in normal Tyrode solution (2.7 mM-K) and on the resting potential at higher potassium concentrations (5.4 , 13.5 , and 50 mM).

3. The rate of diastolic depolarization was increased in acetylglycinate and decreased in nitrate. The action potential was lengthened and the

plateau elevated by large anions; in nitrate the action potential was shortened and the plateau was closer to the resting potential.

4. In potassium-free or potassium- and sodium-free solutions the membrane potential could be changed markedly by an anion disequilibrium. In the potassium- and sodium-free solution it was possible to displace the membrane potential permanently by imposing a new potential difference on the membrane for a few minutes.

5. At membrane potentials between -20 and $+50$ mV the electrical membrane resistance was increased in the acetylglycinate and decreased in the nitrate solution. At membrane potentials between -50 and -100 mV there was no change.

6. The results support the hypothesis that (a) chloride ions are passively distributed, (b) chloride ions contribute but little to the total membrane conductance at the resting potential in normal Tyrode solution (-90 mV), but (c) become important as electrical charge carriers when the membrane is depolarized.

I wish to express my indebtedness to Professor S. Weidmann for his valuable help and criticism during this work. My thanks are also due to Professor A. von Muralt for the hospitality at the Physiological Institute of Berne. My stay in Berne was made possible by a traveller fellowship of the Belgian government and a grant (no. 1138) to Professor S. Weidmann from the Swiss National Science Foundation.

REFERENCES

- ADRIAN, R. H. (1960). Potassium chloride movement and the membrane potential of frog muscle. *J. Physiol.* **151**, 154–185.
- CARMELIET, E. (1959). Effets de la substitution des ions chlorure sur le potentiel de membrane des fibres de Purkinje. *Helv. physiol. acta*, **17**, C18.
- CARMELIET, E. (1960). L'influence de la concentration extracellulaire du K sur la perméabilité de la membrane des fibres de Purkinje de mouton pour les ions ^{42}K . *Helv. physiol. acta*, **18**, C15–C16.
- ETZENSPERGER, J. & BRETONNEAU, Y. (1956). Potentiel consécutif et durée de l'état actif de la fibre musculaire striée. Action des ions NO_3^- , Br^- et I^- . *C.R. Soc. Biol., Paris*, **150**, 1777–1781.
- FALK, G. & LANDA, J. F. (1960). Prolonged response of skeletal muscle in the absence of penetrating anions. *Amer. J. Physiol.* **198**, 289–299.
- GIEBISCH, G., KRAUPP, O., PILLAT, B. & STORMANN, H. (1957). Der Ersatz von extracellulären Natriumchlorid durch Natriumsulfat bzw. Saccharose und seine Wirkung auf die isoliert durchströmte Säugetiermuskulatur. *Pflüg. Arch. ges. Physiol.* **265**, 220–236.
- HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.
- HODGKIN, A. L. & HUXLEY, A. F. (1947). Potassium leakage from an active nerve fibre. *J. Physiol.* **106**, 341–367.
- HOFFMAN, B. F. & CRANFIELD, P. F. (1960). *Electrophysiology of the Heart*. New York: McGraw-Hill Book Company, Inc.
- HUTTER, O. F. & NOBLE, D. (1959). The influence of anions on impulse generation and membrane conductance in Purkinje and myocardial fibres. *J. Physiol.* **147**, 16–17P.
- HUTTER, O. F. & NOBLE, D. (1960). The chloride conductance of frog skeletal muscle. *J. Physiol.* **151**, 89–102.
- HUTTER, O. F. & PADSHA, S. M. (1959). Effect of nitrate and other anions on membrane resistance of frog skeletal muscle. *J. Physiol.* **146**, 117–132.

- LAMB, J. F. (1960). *Electrical activity and ionic gradients in cardiac muscle, with special reference to the effects of 2-4 dinitrophenol*. Ph.D. Thesis, University of Edinburgh.
- LING, G. & GERARD, R. W. (1949). The normal membrane potential of frog sartorius fibres. *J. cell. comp. Physiol.* **34**, 383-406.
- MANERY, J. F. & HAEGE, L. F. (1941). The extent to which radioactive chloride penetrates tissues and its significance. *Amer. J. Physiol.* **134**, 83-93.
- ROBERTSON, W. VAN B. & PEYSER, P. (1956). Estimates of extracellular fluid volume of myocardium. *Amer. J. Physiol.* **184**, 171-174.
- SEKUL, A. A. & HOLLAND, W. C. (1959). Cl^{36} and Ca^{45} exchange in atrial fibrillation. *Amer. J. Physiol.* **197**, 752-756.
- WEIDMANN, S. (1952). The electrical constants of Purkinje fibres. *J. Physiol.* **118**, 348-360.
- WEIDMANN, S. (1955). Rectifier properties of Purkinje fibres. *Amer. J. Physiol.* **183**, 671.
- WEIDMANN, S. (1956). *Elektrophysiologie der Herzmuskelfaser*. Bern: Med. Verlag H. Huber.